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SHORT COMMUNICATION

THE PRESENCE AND PHYTOTOXICITY OF FUMONISINS AND AAL-TOXIN IN *ALTERNARIA ALTERNATA*

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H. K. Abbas and R. T. Riley. The presence and phytotoxicity of fumonisins and AAL-toxin in *Alternaria alternata*. *Toxicon* **34**, 133–136, 1996.—The fumonisins (FB) and AAL-toxin are known to be produced by *Fusarium moniliforme* and *Alternaria alternata* f. sp. *lycopersici* when grown on rice. *Alternaria alternata* produced 1.3–3.1 ppm FB_1 when grown on PDA for 2 weeks at 26°C as determined by CD-ELISA and 0.6 ppm as determined by CF/FAB/MS. ELISA consistently yielded higher results than CF/FAB/MS. The presence of FB_2, FB_3 and AAL-toxin were demonstrated in spores and mycelia of *A. alternata* by CF/FAB/MS. The presence of AAL-toxin was further confirmed by demonstration of increased free sphingoid bases in tomato plants exposed to fungal spores and mycelia. This is the first report of AAL-toxin, FB_2 and FB_3 in spores and mycelia and confirms the presence of FB_1 in *A. alternata*.

The fumonisins and AAL-toxin are related phytotoxins and mycotoxins originally isolated from *Fusarium moniliforme* and *Alternaria alternata* f. sp. *lycopersici*, respectively (Bezuidenhout *et al.*, 1988; Bottini *et al.*, 1981). The fumonisins, including FB_1, FB_2, FB_3, FB_4, FA_1, FA_2 and FC_1, are produced by *F. moniliforme* when grown on solid or liquid media, especially rice and corn (Abbas *et al.*, 1992; Branham and Plattner, 1993; Bezuidenhout *et al.*, 1988). *Alternaria alternata* f. sp. *lycopersici* causes stem canker on susceptible (*asc/asc*) tomatoes (Gilchrist and Grogan, 1976; Kohmoto *et al.*, 1982). AAL-toxin and FB_1 are phytotoxic to *asc/asc* tomatoes and a wide range of other weed and crop species (Abbas and Boyette, 1992; Abbas *et al.*, 1995). Recently, it has been shown that both the fumonisins and AAL-toxins cause disruption of sphingolipid metabolism in susceptible varieties of tomato (Abbas *et al.*, 1994).

It has been demonstrated that AAL-toxin is present in necrotic tissue of infected tomato (Siler and Gilchrist, 1983) as well as by fermentation of *A. alternata* on solid (Abbas and Vesonder, 1993) and liquid media (Bottini *et al.*, 1981). A previous report showed that *A. alternata* can produce FB_1 as well as AAL-toxin (Chen *et al.*, 1992). This report confirms the presence of FB_1 in fungal materials (spores and mycelia) of *F. moniliforme* and *A. alternata* as determined by ELISA and reports for the first time the presence of FB_2, FB_3 and AAL-toxin as confirmed by continuous flow (liquid chromatography) fast atom bombardment/mass spectroscopy (CF/FAB/MS).

The sources of the fungi used in these studies were reported in detail in Abbas and Vesonder (1993) and Abbas *et al.* (1991). Fungal cultures were isolated as single spores and stored on sterile soil or skim milk silica gel. Twenty to 30 Petri plates (9 cm by 1.5 cm) containing 20–25 ml of potato dextrose agar (PDA) or corn meal agar (CMA) were used for each treatment (Abbas *et al.*, 1993). Fungal inoculum of 2 mm^2 was placed in the center of each plate, sealed with parafilm, and incubated under continuous light. After 2 weeks at 26°C for *A. alternata* and 10 days at 22°C for *F. moniliforme*, fungal material was scraped from the surface of the media. Fungal material from approx. 25 plates was combined and weighed (fresh weight). The ability of fresh fungal materials from *A. alternata* (spores and mycelia) to elicit an increase in free sphinganine and free phytosphingosine in tomato was tested essentially the same as previously described for pure toxin and rice culture material (Abbas *et al.*, 1994). Susceptible plants (*asc/asc*) were removed for analysis when symptoms (wilt and necrotic lesions on leaves and stems) developed (20–60 hr). Symptoms never developed in the resistant variety (*Asc/Asc*) which

was sampled 120 hr after exposure to fungal materials. Extraction, purification, and chromatography of free sphingoid bases was the same as described previously (Abbas *et al.*, 1994).

To determine the thermal stability of AAL-toxin and FB, samples of each fungus were autoclaved for 15 min at 121°C and 15 lb/square inch and weighed again. Autoclaved and non-autoclaved fungal material was extracted with acetonitrile:sterile distilled water (1:1, v/v) at 5 ml per 1 g fresh weight and homogenized for 3–5 min in a polytron PT3000 (Brinkmann Instruments, Westbury, NY, U.S.A.). The homogenate was centrifuged for 10 min at 10,000 rpm and evaporated on a rotary evaporator to about 5 or 10 ml. The concentrate was transferred to 15 ml conical tubes for freeze drying.

One-half of the lyophilized extract was used to quantitate FB_1 by Competitive Direct ELISA (CD-ELISA) according to the published procedure by Azcona-Olivera *et al.* (1992). The other half of the lyophilized extract was resuspended in acetonitrile:water (1:1, v/v) and passed through a 0.2 μm pore size millipore filter. An aliquot equivalent to 1 g fungal material was used for confirmation of the presence of the fumonisins and AAL-toxin by CF/FAB/MS as described by Chen *et al.* (1992).

In CD-ELISA, FB_1 was found in *F. moniliforme* spores and mycelia grown on PDA in high concentration (238 and 250 ppm) (Table 1). FB_1 was also found in spores and mycelia of *A. alternata* grown on PDA in low concentration (1.3 and 3.1 ppm) (Table 1). Chen *et al.* (1992) reported that FB_1 was found in *A. alternata* grown on liquid media. This study provides confirmatory evidence that FB_1 and AAL-toxin can be produced by the same fungus (Table 1). The presence of FB_1 was confirmed by CF/FAB/MS at 17 ppm in fungal material from *F. moniliforme* and at 0.6 ppm in fungal material from *A. alternata*. Concentrations of FB_1 by CD-ELISA were consistently higher than those found in CF/FAB/MS. This finding is in agreement with previous results of determination of FB_1 by ELISA vs. HPLC as recently reported by Tejada-Simon *et al.* (1995). FB_2 and FB_3 were also found in *F. moniliforme* at approximately 0.3 ppm each by FC/FAB/MS. FB_2 and FB_3 were also present in trace amounts, about 0.05 ppm, in *A. alternata*, suggesting that the fumonisin biosynthetic pathway is present in both fungi.

Specific ELISA antibodies are not yet available for AAL-toxin. Therefore, CF/FAB/MS was the only method used for its measurement. AAL-toxin was found in *A. alternata* at 0.5 ppm. It was not found in the *F. moniliforme* spore and mycelia extracts. This is the first report of AAL-toxin in spores and mycelia of *A. alternata*. Also, the autoclaving process did not destroy FB_1. This finding is in agreement with previous results on stability of FB_1 to temperature which was reported by Alberts *et al.* (1990). AAL-toxin was also shown to be heat-stable, which confirms the finding of Lamprecht *et al.* (1994) and Gilchrist and Grogan (1976).

The relationship of AAL-toxin to the symptoms of *A. alternata* stem canker on tomato has not been confirmed. Symptoms caused by *A. alternata in vivo* are similar to those caused by pure AAL-toxin lending support to this finding (Abbas *et al.*, 1994; Kohmoto *et al.*, 1982). Application of spore and mycelia suspensions to susceptible and resistant tomato plants caused increase in free sphingasine and phytosphingosine concentrations qualitatively similar (Table 2) to those obtained using pure AAL-toxin (Abbas *et al.*, 1994). This group of toxins has important phytotoxicity to weed and crop plants. This study expands our knowledge of the presence of these toxins in these pathogenic fungi.

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Table 1. Fungi used in these studies and fumonisin B_1 concentration determined by CD-ELISA

Treatment*	Growth media	Fungal material (g)	Incubation period (day)	FB_1† concentration (ppm)
Non-autoclaved *F. moniliforme*	PDA	16.4	10	250 ± 5
Autoclaved *F. moniliforme*	PDA	16		
Non-autoclaved *A. alternata*	PDA	8		
Autoclaved *A. alternata*	PDA	6		
Non-autoclaved *A. alternata*	CMA	7		
Autoclaved *A. alternata*	CMA	6.4	16	0.1 ± 0.1
LSD ($P = 0.05$)			19.7	

*Non-autoclaved: fresh fungal material (spores and mycelia); autoclaved: dead fungal material (spores and mycelia); *F. moniliforme* NRRL 18738 isolated from jimsonweed in MS; and *A. alternata* NRRL 18822 isolated from tomato plants in MS.

†The values are the results of two replicates ± S.D.

Table 2. Concentration (pmol/mg dry weight) of free phytosphingosine and free sphinganine in extracts from susceptible (*asc/asc*) and resistant (*Asc/Asc*) tomato plants treated with either water (control) or suspensions of fungal materials (spores and mycelia) of *A. alternata*

Treatment	Phytosphingosine	Sphinganine
(*asc/asc*)		
Controls ($n = 12$)	4–17	2–10
Treated*		
0.5×10^5 ($n = 4$)	65–296	152–483
(*Asc/Asc*)		
Control ($n = 7$)	3–5	1–2
Treated*		
0.5×10^5 ($n = 2$)	96–115	12–15

*The range of values for each treatment is shown. n is the number of plants.

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